

ACKNOWLEDGMENTS

We acknowledge Joseph DiMaria for excellent technical support.

**Qian Zheng¹, Siming Chen¹,
Ying Chen¹, John Lyga¹ and
Uma Santhanam¹**

¹Global Research and Development, Avon
Products Inc., Suffern, New York, USA
E-mail: qian.zheng@avon.com

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at <http://www.nature.com/jid>

REFERENCES

- Caltagarone J, Hamilton RL, Murdoch G *et al* (2010) Paxillin and hydrogen peroxide-inducible clone 5 (Hic-5) expression and distribution in control and Alzheimer disease hippocampus. *J Neuropathol Exp Neurol* 69:356–71
- Deakin NO, Turner CE (2008) Paxillin comes of age. *J Cell Sci* 121:2435–44
- Fisher GJ, Varani J, Voorhees JJ (2008) Looking older: fibroblast collapse and therapeutic implications. *Arch Dermatol* 144:666–72
- Hagel M, George EL, Kim A *et al* (2002) The adaptor protein paxillin is essential for normal development in the mouse and is a critical transducer of fibronectin signaling. *Mol Cell Biol* 22:901–15
- Hao Q, Rutherford SA, Low B *et al* (2006) Selective regulation of hydrogen peroxide signaling by receptor tyrosine phosphatase- α . *Free Radic Biol Med* 41:302–10
- Karaköse E, Schiller HB, Fässler R (2010) The kindlins at a glance. *J Cell Sci* 123:2353–6
- Lee TY, Gotlieb AI (1999) Early stages of endothelial wound repair: conversion of quiescent to migrating endothelial cells involves tyrosine phosphorylation and actin microfilament reorganization. *Cell Tissue Res* 297:435–50
- Nishio K, Inoue A (2005) Senescence-associated alterations of cytoskeleton: extraordinary production of vimentin that anchors cytoplasmic p53 in senescent human fibroblasts. *Histochem Cell Biol* 123:263–73
- Qin J, Vinogradova O, Plow EF (2004) Integrin bidirectional signaling: a molecular overview. *PLoS Biol* 2:734–29
- Varani J, Dame MK, Rittie L *et al* (2006) Decreased collagen production in chronologically aged skin: roles of age-dependent alteration in fibroblast function and defective mechanical stimulation. *Am J Pathol* 168:1861–8
- Varani J, Schuger L, Dame MK *et al* (2004) Reduced fibroblast interaction with intact collagen as a mechanism for depressed collagen synthesis in photodamaged skin. *J Invest Dermatol* 122:1471–9
- Webb DJ, Brown CM, Horwitz AF (2003) Illuminating adhesion complexes in migrating cells: moving toward a bright future. *Curr Opin Cell Biol* 15:614–20
- Wolfenson H, Henis YI, Geiger B *et al* (2009) The heel and toe of the cell's foot: a multifaceted approach for understanding the structure and dynamics of focal adhesions. *Cell Motil Cytoskeleton* 66:1017–29
- Zamir E, Geiger B (2001) Molecular complexity and dynamics of cell-matrix adhesions. *J Cell Sci* 114:3583–90
- Zhou L, Li Y, Yue BY (1999) Oxidative stress affects cytoskeletal structure and cell-matrix interactions in cells from an Ocular tissue: The trabecular meshwork. *J Cell Physiol* 180:182–9

Histone Deacetylase 2 Is Upregulated in Normal and Keloid Scars

Journal of Investigative Dermatology (2012) **132**, 1293–1296; doi:10.1038/jid.2011.432; published online 29 December 2011

TO THE EDITOR

Repair following cutaneous injury is essential to return function, form, and integrity to the tissue. Scar formation is the inevitable and currently unpreventable consequence of tissue damage, and misregulation can lead to the development of pathological scarring as in hypertrophic and keloid scars. In developed countries alone, approximately 100 million people each year will be left with a scar following surgery; moreover, there are thought to be 11 million people with keloid scars (Sund, 2000). This represents a significant burden to both health-care providers and individuals.

Currently, the pathogenesis of keloid scars is not fully understood, but there are a number of clues as to the etiology. There is an obvious genetic component,

and they have also been linked to hormonal influences, wound infection, and skin tension (Wolfram *et al.*, 2009). Given the burden presented and our current poor ability to reduce or prevent normal and pathological scarring, clearly an improved understanding of the molecular mechanisms underlying scar formation is essential, and research into novel treatment strategies is justified.

Histone deacetylases (HDACs) and counteracting histone acetyltransferases (HATs) are epigenetic modifying enzymes that are accepted to, respectively, remove and add acetyl groups to histones, and in this manner influence gene expression (acetylation is generally an activating, transcription-promoting modification; Kuo and Allis, 1998). The objective of this work was to

characterize the expression profiles of specific HDACs in normal and keloid scars; this has not been previously investigated, and there is a lack of consensus about the role of histone (de)acetylation in various fibrotic conditions. For example, HDAC inhibitors used *in vitro* suppressed myofibroblast differentiation (Glenisson *et al.*, 2007; Mannaerts *et al.*, 2010) and decreased collagen production in keloid fibroblasts (Diao *et al.*, 2011); in animal models, HDAC inhibition decreased heart, kidney, and liver fibrosis (Pang *et al.*, 2009; Iyer *et al.*, 2010; Marumo *et al.*, 2010). Conversely, HATs were overexpressed in fibrotic lesions of scleroderma patients (Bhattacharyya *et al.*, 2005), and HAT inhibition was found to be anti-fibrotic (Li *et al.*, 2008).

To investigate HDAC expression in human skin scars, three types of skin tissue were assessed by immunohistochemistry (IHC): normal human skin, normal scar

Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HAT, histone acetyltransferase; HDAC, histone deacetylase; IHC, immunohistochemistry; TGF β , transforming growth factor- β

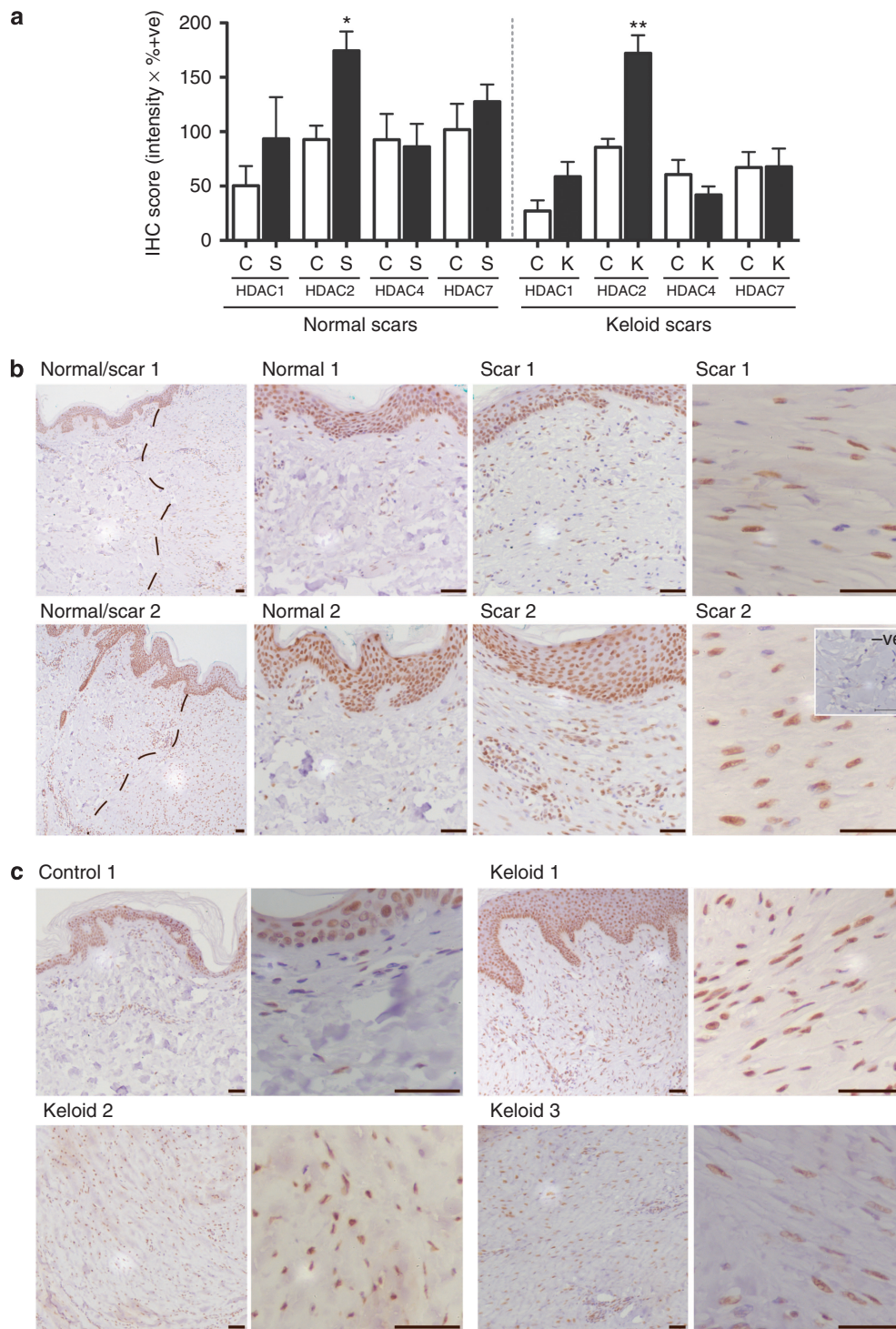


Figure 1. Immunohistochemistry (IHC) reveals histone deacetylase (HDAC)2 upregulation in human scar tissue. (a) Formalin-fixed, paraffin-embedded human scar samples were analyzed by IHC for expression of HDAC1, 2, 4, and 7. At least three independent reviewers scored the staining intensity and the percentage of positive dermal fibroblast cells in normal skin and scar tissue. The product of these two numbers (mean of 3/4 scorers) gave the IHC score, which was statistically analyzed and is graphically represented. Results were analyzed by paired *t*-tests (normal scars) or unpaired *t*-tests (keloids); *, *P* < 0.005 (*n* = 10); **, *P* < 0.0001 (*n* = 11). (b, c) Representative patient samples showing HDAC2 upregulation in (b) normal scar tissue, and (c) keloid scars are shown. Brown staining indicates HDAC2 positivity, omission of the primary antibody served as a negative control. Bars = 50 μm.

tissue from patients undergoing melano-
ma re-excision approximately 2–3 weeks
after the original wound, and keloid scar

tissue from revision procedures (age
of scar > 6 months). Tissue samples were
batch analyzed for HDAC1, HDAC2,

HDAC4, and HDAC7. Scar-associated
fibroblasts in both normal and keloid
scars showed a significant and striking

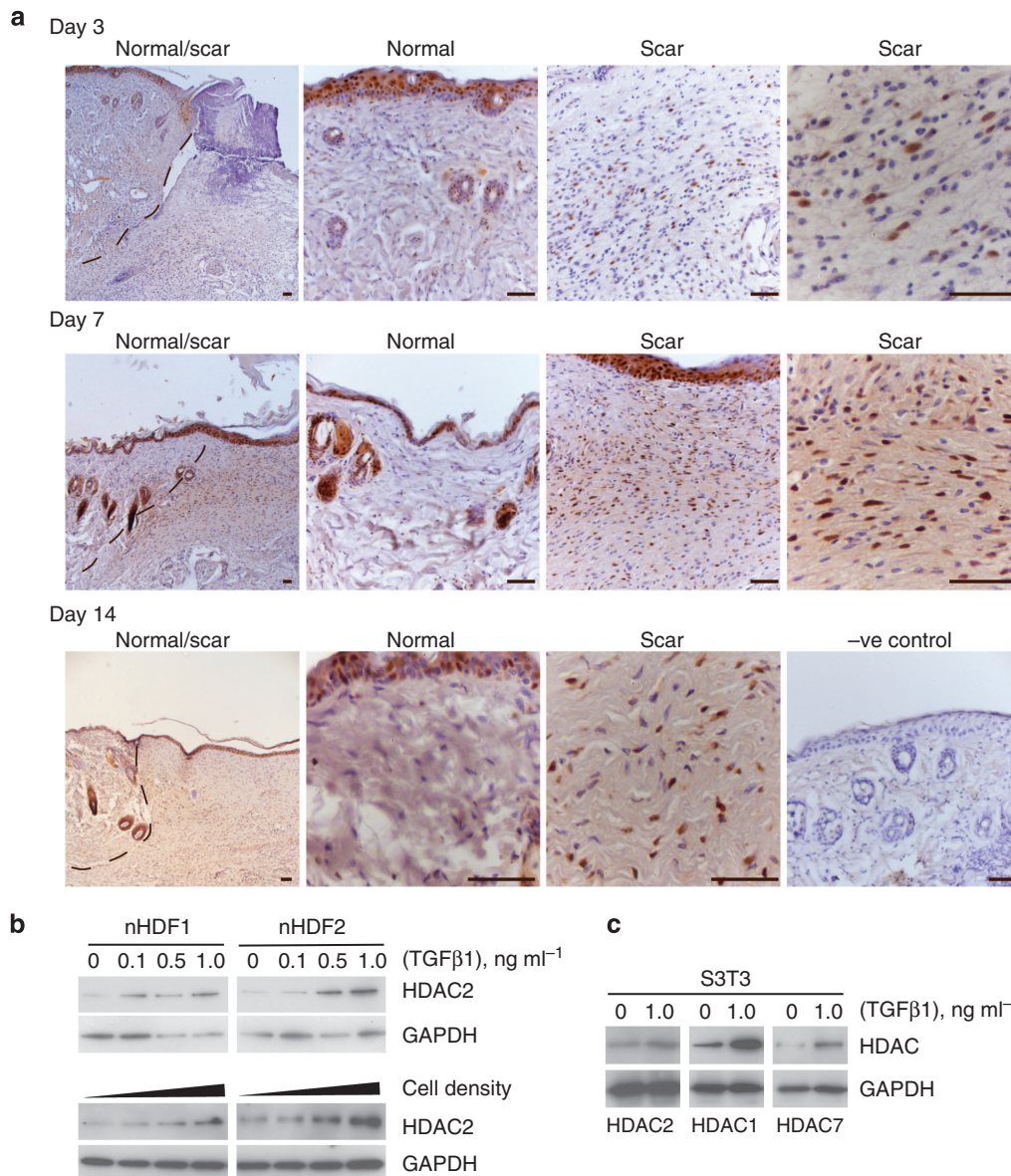


Figure 2. Transforming growth factor (TGF)β1 and cell density may regulate histone deacetylase (HDAC2) expression in scar tissue. (a) Immunohistochemistry of scars resulting from a 4-mm punch biopsy wound made to the dorsal skin of adult male mice showed elevated HDAC2 expression (brown) in the scar-associated fibroblasts throughout the time course of scar formation. Omission of the primary antibody served as a negative control. Bars = 50 μm. (b) Western blot analysis of normal human dermal fibroblasts (nHDF, < passage 15) treated with the indicated concentrations of TGFβ1 for 24 h, or cultured at varying densities (approximately 25, 50, 75, and 100% confluence) for 96 h, revealed a "concentration"-dependent increase in HDAC2 expression. (c) TGFβ1 (1 ng ml⁻¹) stimulation of Swiss 3T3 (S3T3) cells also resulted in upregulation of HDAC2, as well as an increase in HDAC1 and 7. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) served as a loading control.

upregulation of HDAC2, but not HDAC1, 4, or 7 (Figure 1). Within the epidermis, HDAC expression was unchanged in scar versus non-scar regions (data not shown).

Our observation that HDAC2 was upregulated in scar tissue was substantiated using a mouse model of wound repair. Specifically, 4-mm excisional wounds were made to the shaved

dorsal skin of anesthetized adult male mice (CD-1; 6–8 weeks; protocol approved by an institutional ethics committee and the UK Home Office). Wound tissue was harvested after 3, 7, or 14 days and analyzed for HDAC2 by IHC. On Day 3, HDAC2-positive cells were found at the wound margins, whereas on Days 7 and 14 highly expressing cells were abundant in the

wound bed (Figure 2a). This approach demonstrated that HDAC2 is upregulated in normal scar tissue in the mouse as it is in human.

To begin to understand how HDAC2 expression may be regulated in this setting, primary cultures of normal human dermal fibroblasts (nHDFs, < passage 15) were either (1) treated with transforming growth factor

(TGF) β 1 (0, 0.1, 0.5, 1 ng ml⁻¹) or (2) cultured at varying densities, and the effects on HDAC2 expression were observed by western blot analysis. TGF β 1 was able to trigger a concentration-dependent upregulation of HDAC2 (Figure 2b). Intriguingly, this effect was not observed in all patients: only two of the four primary cell populations tested responded in this way. Future work will investigate the determinants of this variable response and its influence on the efficacy of different targeted therapeutics. The same two nHDF isolations showed that HDAC2 expression correlates positively with cell density (Figure 2b), which would be high in a developing scar *in vivo*. Experiments using murine Swiss 3T3 fibroblasts (< passage 12) confirmed that TGF β 1 can increase HDAC2 expression, and, interestingly, also revealed that HDAC1 and HDAC7 were TGF β 1-responsive in these cells (Figure 2c). This seemingly contradictory finding that only HDAC2 was upregulated in *in vivo* scars, whereas TGF β 1 stimulation of cultured fibroblasts increased HDAC1 and 7, as well as 2, may indicate that the timing and duration of TGF β 1 exposure are important factors in the regulation of specific family members; alternatively, there may be confounding negative influences on the expression of specific HDACs in the heterogeneous *in vivo* setting.

Our *in vivo* studies on mouse and human skin wounds revealed that HDAC2 is significantly overexpressed in both normal and keloid scar tissue. The failure of HDAC2 to distinguish between normal and keloid scars leads us to speculate that, in keloids, expression may fail to return to normal levels at the completion of the healing process, and thus may contribute to persistent growth and/or alternative differentiation of these cells.

Our ongoing hypothesis is that pharmacological inhibition of HDACs will decrease skin fibrosis. Early, but promising, findings in various animal models of fibrosis certainly indicate that this may be the case in other organ

systems (Pang *et al.*, 2009; Iyer *et al.*, 2010; Marumo *et al.*, 2010). As inflammation tends to exacerbate scarring, it will be interesting to determine whether these results are owing to HDAC inhibitors acting directly on the fibroblasts, or acting indirectly, as anti-inflammatories (Han and Lee, 2009). Regardless of the mode of action, if correct, the pathway from bench to bedside for HDAC inhibitors in the treatment of skin scars is anticipated to be relatively smooth, as there are 13 HDAC inhibitors already in use clinically (Paris *et al.*, 2008).

Currently, there are no successful treatments that prevent or eliminate scar tissue; however, counteracting TGF β 1, for example, using recombinant TGF β 3 (Avotermin, Renovo, UK), has shown potential success as an anti-scarring treatment (So *et al.*, 2011). Our finding that TGF β 1 treatment increased HDAC expression suggests that HDAC inhibitors equally have the potential to be anti-fibrotic, depending on the extent to which TGF β 1 relies on HDAC2 to exert its effects. People with scars face many physical, psychological, esthetic, and social consequences that may be associated with substantial emotional and financial cost (Brown *et al.*, 2008). This research implicates HDACs in skin scarring, and suggests they may be novel therapeutic targets for the prevention of normal and pathological scarring.

CONFLICT OF INTEREST

The authors state no conflict of interest.

ACKNOWLEDGMENTS

This work was supported by the University of London Central Research Fund and St George's, University of London.

**Edmund J. Fitzgerald O'Connor¹,
Irbaz I. Badshah², Lucy Y. Addae²,
Preeya Kandasamy², Sukanthini
Thanabalasingam², Daniel Abioye²,
Mark Soldin¹ and Tanya J. Shaw²**

¹Department of Plastic and Reconstructive Surgery, St George's Healthcare NHS Trust, London, UK and ²Division of Biomedical Sciences, St George's University of London, London, UK.
E-mail: tshaw@sgul.ac.uk

REFERENCES

- Bhattacharyya S, Ghosh AK, Pannu J *et al.* (2005) Fibroblast expression of the coactivator p300 governs the intensity of profibrotic response to TGF β . *Arthritis Rheum* 52: 1248–58
- Brown BC, McKenna SP, Siddhi K *et al.* (2008) The hidden cost of skin scars: quality of life after skin scarring. *J Plast Reconstr Aesthet Surg* 61:1049–58
- Diao JS, Xia WS, Yi CG *et al.* (2011) Trichostatin A inhibits collagen synthesis and induces apoptosis in keloid fibroblasts. *Arch Dermatol Res* 303:573–80
- Glenisson W, Castronovo V, Waltregny D (2007) Histone deacetylase 4 is required for TGF β -induced myofibroblastic differentiation. *Biochim Biophys Acta* 1773:1572–82
- Han SB, Lee JK (2009) Anti-inflammatory effect of Trichostatin-A on murine bone marrow-derived macrophages. *Arch Pharm Res* 32:613–24
- Iyer A, Fenning A, Lim J *et al.* (2010) Antifibrotic activity of an inhibitor of histone deacetylases in DOCA-salt hypertensive rats. *Br J Pharmacol* 159:1408–17
- Kuo MH, Allis CD (1998) Roles of histone acetyltransferases and deacetylases in gene regulation. *Bioessays* 20:615–26
- Li HL, Liu C, de Couto G *et al.* (2008) Curcumin prevents and reverses murine cardiac hypertrophy. *J Clin Invest* 118:879–93
- Mannaerts I, Nuytten NR, Rogiers V *et al.* (2010) Chronic administration of valproic acid inhibits activation of mouse hepatic stellate cells *in vitro* and *in vivo*. *Hepatology* 51:603–14
- Marumo T, Hishikawa K, Yoshikawa M *et al.* (2010) Histone deacetylase modulates the proinflammatory and -fibrotic changes in tubulointerstitial injury. *Am J Physiol Renal Physiol* 298:F133–41
- Pang M, Kothapally J, Mao H *et al.* (2009) Inhibition of HDAC activity attenuates renal fibroblast activation and interstitial fibrosis in obstructive nephropathy. *Am J Physiol Renal Physiol* 297:F996
- Paris M, Porcelloni M, Binaschi M *et al.* (2008) Histone deacetylase inhibitors: from bench to clinic. *J Med Chem* 51:1505–29
- So K, McGrouther DA, Bush JA *et al.* (2011) Avotermin for scar improvement following scar revision surgery: a randomized, double-blind, within-patient, placebo-controlled, phase II clinical trial. *Plast Reconstr Surg* 128(1):163–72
- Sund B (2000) *New Developments in Wound Care*. London: PJB Publications
- Wolfram D, Tzankov A, Pulzl P *et al.* (2009) Hypertrophic scars and keloids—a review of their pathophysiology, risk factors, and therapeutic management. *Dermatol Surg* 35: 171–81